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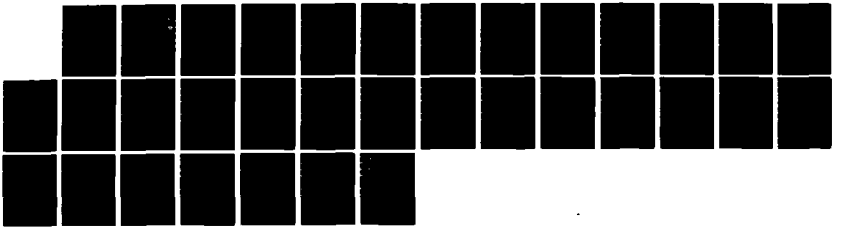

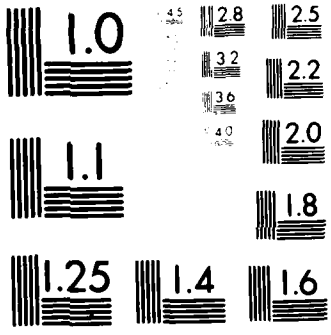

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19. ABSTRACT (Continue on reverse if necessary and identify by block number)

During this reporting period, further examples of 1,1-dimethylhydrazine (UDMH) induced immunoenhancement have been noted, and at least two possible mechanisms for immunoenhancement have been suggested. First, in vivo or in vitro exposure to UDMH results in enhancement of the allogeneic mixed lymphocyte response, a measure of cell mediated immunity. Interference of certain macrophage functions by UDMH has been demonstrated, such as decreased prostaglandin E_2 production and chemiluminescence. Inhibition of these properties which are associated with macrophage-related immunosuppression could explain the immunoenhancement induced by UDMH. Preliminary experiments also suggest that UDMH enhances interleukin 1 production by macrophages, and interleukin 2 activity on cell proliferation, but that it inhibits interleukin 1 activity.

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Final Report

IMMUNE DYSFUNCTIONS AND ABROGATION OF THE INFLAMMATORY RESPONSE BY ENVIRONMENTAL CHEMICALS

Richard G. Olsen
Department of Veterinary Pathobiology

For the Period
July 1, 1984 - December 31, 1985

AIR FORCE OFFICE OF SCIENTIFIC RESEARCH
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I. Research Objectives

During the past 1-1/2 years (7/1/84-12/31/86) several previously stated objectives have been achieved, and some new objectives were established and either have been or are in the process of being met. All experiments have been designed to determine possible mechanisms of immunomodulation exerted by 1,1-dimethyl-hydrazine (UDMH). Previously proposed specific aims which have been accomplished or nearly accomplished include:

A. Evaluation of the in vitro effects of chemiluminescence in activated macrophages.

B. Evaluation of the in vivo effects of UDMH on murine allogeneic mixed lymphocyte response.

C. Evaluation of the in vitro effects of UDMH on interleukin 1 (IL1) and interleukin 2 (IL2) production and activity.

Additional experiments which have been completed or just initiated:

D. Determination of the in vivo effects of UDMH on Corynebacterium parvum induced immunosuppression.

E. Evaluation of the effects of UDMH exposure on the immunosuppressive properties of activated macrophages.

F. Evaluation of the effects of UDMH on hydrogen peroxide-induced suppression of lymphoproliferation.

Proposed specific aims which have not been completed were:

G. Evaluation of the in vitro effects of UDMH on T-lymphocyte antigens (Lyt 1 and Lyt 2, and Thy) and HLA antigen (Ia). These experiments were deferred until the acquisition of the flow cytometer. They should be completed within the next year.

H. Evaluation of the interaction of UDMH with soluble immune response

suppressor (SIRS). These experiments have been deferred until a SIRS-producing hybridoma can be created or obtained.

II. Status of Research:

A. Summary of significant results during FY 1983-1984 (excerpted from Progress Report for July 1, 1983 - June 30, 1984): Results of two sets of experiments indicate that the macrophage was a target cell of UDMH-induced immunomodulation. First, when UDMH was present in the medium during the allogeneic mixed lymphocyte reaction (MLR), the response was enhanced at lower concentrations. The enhancement was greater and present at all concentrations when the stimulator cell population, which was enriched for macrophages, was preincubated with UDMH for two hours before being washed and added to the responding cell population for the MLR assays. On the other hand, when the responding cell population, which was enriched for T cells, was preincubated with UDMH, no enhancement was noted (see Table 1).

Secondly, it was determined that UDMH reduced prostaglandin E_2 (PGE_2) production by resident peritoneal macrophages (Figure 1). This finding corroborated earlier experiments which showed that exposure to UDMH resulted in decreased PGE_2 production by adherent splenocytes (a macrophage-enriched cell population), and suggested a possible mechanism for UDMH-induced immunoenhancement. Since PGE_2 is immunosuppressive, and is considered to play a significant role in normal immunoregulation, reduction of PGE_2 by UDMH could interfere with this immunoregulatory system and be manifest by immunoenhancement. Other compounds which interfere with PGE_2 synthesis, such as indomethacin, also cause immunoenhancement.

Other experiments completed during 1983-1984 revealed that UDMH: 1) caused enhancement of the syngeneic mixed lymphocyte response; 2) did not affect lymphocyte receptor mobility ("capping"); 3) did not affect lymphocyte cyclic nucleotide levels; and 4) had equivocal effects on lymphocyte and macrophage membrane antigens (experiments are to be repeated using the flow cytometer).

Following is a more detailed summary of work completed from July 1, 1984 to Dec. 31, 1985 (termination of contract no. F49620-83-C-0014).

B. Effects of UDMH on chemiluminescent response of activated macrophages.

1. Rationale:

Since many previous experiments have indicated that UDMH suppresses some macrophage functions, these experiments were designed to further characterize the effects of UDMH on macrophage function. Chemiluminescence is a phenomenon which occurs when many cell types are exposed to particular stimuli. When activated macrophages are exposed to stimuli such as opsonized particles or certain chemicals (e.g. phorbol esters), they undergo a "respiratory burst" response which results in the release of highly reactive oxygen metabolites. "Chemiluminescence" refers to the light released when this occurs, and it can be measured in a scintillation counter or luminometer. One of the oxygen products produced, hydrogen peroxide (H_2O_2), closely corrrelates with the degree of chemiluminescence. Theoretically, the immunoenhancement effects of UDMH could be explained by interference of H_2O_2 production, since H_2O_2 is immunosuppressive itself, and it is also

necessary to activate soluble immune response suppressor or "SIRS", a suppressive lymphokine.

2. Methods:

Balb/c female mice were injected intraperitoneally with 1.4 mg killed Corynebacterium parvum (C. parvum), a macrophage activating bacterium. Seven days after injection, the activated peritoneal macrophages (APM) were harvested, washed and mixed with luminol (a chemical which enhances the intensity of the light produced by the cells), colorless culture medium with or without UDMH, and zymosan particles which were opsonized by fresh horse serum. The vials containing this mixture were placed in a liquid scintillation counter (set in the "off-coincidence" mode with the front photomultiplier tube turned off), and counts per minute (cpm) were recorded every five minutes for 45 minutes. The peak response in cpm and time of peak response was recorded for each vial.

3. Results:

UDMH caused suppression of the chemilumnescent response at concentrations of 5-100 µg/ml. There was a concentration-dependent suppression at 5, 10 and 25 µg/ml, then the cpm remained between 25% and 35% of control values for 25, 50, 75 and 100 µg/ml (see Figure 1). Viability of the macrophages was not affected by the UDMH.

The time of the peak response was not significantly altered by UDMH except at 75 and 100 µg/ml when the peak response occurred at an earlier time (Table 2).

4. Significance:

Interference of macrophage chemiluminescence lends support to the premise that a major mechanism of UDMH-induced

immunoenhancement is abrogation of (activated) macrophage function. Future experiments have been proposed to determine if UDMH suppresses macrophage function by interference with H_2O_2 production.

B. In vivo effects of UDMH on murine allogeneic mixed lymphocyte response (MLR).

Earlier experiments indicated that if UDMH was added to MLR cultures, an enhancement of the response was seen under various conditions. A second set of experiments was designed to determine if similar effects were noted with in vivo UDMH exposure. All mice were injected intraperitoneally (i.p.) daily for 7 days with PBS (for control) or UDMH (5, 10, 25, 50, 75 or 100 mg/kg). Three types of experiments were done: Responder cells from UDMH-treated mice (C57Bl/6) were cultured with stimulator cells from untreated mice (Balb/c); Responder cells from untreated mice were cultured with stimulator cells from UDMH-treated mice; and both responder and stimulator cells from UDMH-treated mice were cultured together. Responder cells were enriched for T-cells by passage through a nylon wool column; stimulator cells were enriched for B-cells and macrophages by centrifugation through a bovine serum albumin gradient. The cultures were incubated for 4 days; tritiated thymidine was added during the last 18 hours of culture. The cells were then harveted using a multiple automated sample harvester, and the proliferative response was expressed in counts per minute.

The results of one experiment are shown in Table 3. All combinations of cells at nearly all doses of UDMH resulted in enhanced MLR responses. Two other experiments showed similar results, although the dose-response patterns were slightly different.

These results would suggest that both T-lymphocytes (responder cell population), and B-lymphocytes and macrophages (stimulator population) may be affected in animals exposed to UDMH, with a resultant enhancement in the immune response. In contrast, in the in vitro experiments the MLR was not enhanced when responder cells (T-lymphocytes) were pre-incubated with UDMH, but only when stimulator cells or both populations were exposed to UDMH. This discrepancy is most likely related to the method of UDMH exposure (in vitro versus in vivo).

C. Effects of UDMH on IL1 production and activity.

1. Rationale: The macrophage-derived protein, interleukin 1 (IL-1), appears to play a critical role in immunological responses. In vitro IL-1 has been found responsible for regulating T-cell proliferation by inducing interleukin 2 (IL-2) production by T-lymphocytes, and enhancing B-cell differentiation and antibody secretion. Fever induction, stimulation of acute-phase protein synthesis and induction of neutrophilia are some in vivo properties of IL-1. Agents altering the ability of macrophages to synthesize and secrete IL-1 would undoubtedly modulate the immune system due to multiple IL-1 functions. Since many of our previous experiments indicate that the macrophage is a target cell for UDMH-induced immunomodulation, the effects of UDMH on IL-1 production and activity are currently being evaluated.

2. Methods: The cell source of IL1 was an adherent macrophage cell line, $P388_{D1}$. Cells were grown to confluency, then cultured with phorbol myristate acetate (PMA) for 5 hours, then washed and further cultured in the presence of fresh medium for 24 hours. The supernatant was harvested and assayed for IL1 activity using thymocytes from C3H/HeJ mice. The thymocytes were cultured with

phytohemagglutinin (pha) and Il1 supernatant for 72 hours, and then evaluated for proliferative activity by measurement of tritiated thymidine uptake.

3. Results:

a. Effects of UDMH on Il-1 production: UDMH was added to P388 cultures either during (t = 0-5 hrs) or following (t = 5-29 hrs) incubation with PMA. When added at t = 0-5 hrs, UDMH did not have any consistent significant effect on IL-1 production (Table 3); however, when added at t = 5-29 hrs, UDMH augmented IL-1 production at 25, 50 and 100 ug/ml (Table 4).

b. Effects of UDMH on IL-1 activity: UDMH was added to the C3H/HeJ thymocytes at the beginning of the incubation time, simultaneously with pha and the IL-1-containing supernatant from normal $P388_{D1}$ cells. Results from preliminary experiments show a significant dose-related suppression of the proliferative response of the thymocytes in the presence of UDMH (Table 5). This suppression was not due to UDMH-induced cytotoxicity (Table 5).

4. Significance: See section D4.

D. Effects of UDMH on IL-2 production and activity.

1. Rationale: Interleukin 2 (IL-2), previously referred to as T-cell replacing factor, is a lymphokine produced by the Lyt. 1^+, 2^-, 3^- T-cell (helper T-cell) population following antigen or mitogen stimulation. In vitro, IL-2 has been shown to increase antibody synthesis, promote cytotoxic T-cell proliferation and allow the maintenance of long-term T-cell culture lines. In vivo administration of IL-2 in mice produced accelerated allograft rejection and regression of established tumors. Based on its

immunoenhancing properties, IL-2 is now being examined as a possible immunotherapeutic agent.

Although the macrophage appears to be the primary target cell of UDMH-associated immunomodulation, hence IL-1 would be more likely altered, it is still important to evaluate the effects of UDMH on T-cell IL-2 production and activity, since there may be multiple mechanisms of action of UDMH, and the assays for IL-2 production and activity are currently in use in our laboratory for other projects.

2. Methods: Two sources of IL-2 were used. One was splenocytes from normal mice, which were cultured with 1.6 µg/ml concanavalin A (to stimulate IL-2 production) for 24 hours; the supernatant was then harvested for IL-2 activity (see below). The second source of IL-2 was a murine helper T-cell line, EL-4, cultured in the presence of phorbol myristate acetate; the supernatant was harvested after 24 hours and assayed for IL-2 activity. IL-2 activity was assayed using a murine T-cell line CTLL-20, which is dependent on IL-2 for proliferation following stimulation by phytohemagglutinin (pha). The culture medium contained pha and 5% IL-2-containing supernatant (test as well as a standard control). After 24 hrs of incubation, CTLL-20 proliferation was evaluated by measurement of tritiated thymidine incorporation.

3. Results:

a. Effects of UDMH on IL-2 production: For these experiments, UDMH was added to either splenocytes or EL-4 cells simultaneously with the stimulating substance (con A or PMA, respectively. After 24 hours of incubation, the supernatants were harvested and assayed for IL-2 activity on normal CTLL-20 cells. Preliminary results

indicate that UDMH does not affect IL-2 production by spleen cells (Table 6), although further experiments are necessary to confirm this observation. Experiments using EL-4 as the IL-2 source have been completed, but the raw data has not as yet been processed so the results are unavailable.

b. Effects of UDMH on IL-2 activity: For these experiments, CTLL-20 cells were incubated with a standardized source of IL-2, pha, and UDMH, and then evaluated for proliferation after 24 hours. Results of a preliminary experiment indicate that UDMH may enhance IL-2 activity at lower concentrations (5-25 ug/ml), but suppress it at higher concentrations (35-50 ug/ml) (Table 7). The decrease in IL-2 activity at higher concentrations was not due to inactivation of the IL-2 molecule by the UDMH molecule in that preincubation of IL-2-containing medium with UDMH for 24 hours did not alter the IL-2 activity (Table 8).

4. Significance, IL-1 and IL-2 experiments:

At this stage it is unrealistic to extrapolate results from these preliminary experiments, using uniform cell lines, to the whole animal. However, two effects of UDMH which have so far been noted, i.e. enhancement of IL-1 production and enhancement of IL-2 activity, are compatible with augmentation of the immune response which is induced by UDMH. Of particular interest is the enhancement of IL-2 activity at low concentrations of UDMH, because immunoenhancement was most notable at low UDMH exposure levels (both in vitro and in vivo) in many of the earlier experiments. If the present observation holds true, further experiments are planned to determine the effects of UDMH on IL-2 receptor expression, and on cell proliferation which is not dependent on IL-2.

The inhibitory effect of UDMH on IL-2 activity at somewhat higher concentrations would not be compatible with overall immunoenhancement. However, results of certain other experiments in which UDMH was present during rapid lymphocyte proliferation (e.g. lymphocyte blast transformation, mixed lymphocyte responses), as is the case with the CTLL-20 cells, indicate that UDMH does suppress the lymphoproliferation response at similar concentrations (> 25 μg/ml). This suppression has not been related to overt cytotoxicity.

The other effect of UDMH which conflicts with its immunoenhancement properties is inhibition of IL-1 activity. At present, the significance of this observation is unknown.

E. Effects of UDMH treatment on Corynebacterium parvum induced suppression.

1. Rationale: It has been reported that administration of Corynebacterium parvum (C. parvum) to mice results in suppression of various immune functions (Scott, Cell Immunol 5:459, 1972a) and that this immunosuppression is due mainly to macrophage activation (Scott, Cell Immunol 5:469, 1972b). Since UDMH interferes with other activated macrophage properties (e.g. chemiluminescence), it was of interest to determine if UDMH had any effect on C. parvum-induced immunosuppression.

2. Methods. Female Balb/c mice were injected i.p. with 1.4 mg C. parvum. On the same day, and daily for 6 days thereafter, they were also injected i.p. with either phosphate buffered saline (for control) or 25, 50 or 100 mg/kg UDMH. The mice were sacrificed on the day after their last injection and their spleen cells were

assayed for lymphocyte blast transformation (LBT) response to concanavalin A (con A) and for IL-2 production. A group of mice without any treatment served as a normal control. The LBT response was determined by measurement of tritiated thymidine uptake by splenocytes after 24 and 48 hours; IL-2 production was determined by the CTLL-20 proliferation assay (as described above).

3. Results:

a. Effects of UDMH treatment on the LBT response of C. parvum-injected mice.

The combined UDMH-C. parvum treatment resulted in partial reversal of the suppressed LBT response to con A compared to C. parvum treatment alone. After 24 hours, the LBT response was restored to control (untreated mouse) levels in all UDMH treatment groups; after 48 hours, the LBT response of all UDMH treatment groups was significantly greater than that of C. parvum-treated mice, but significantly less than that of untreated mice (Table 9).

b. Effect of UDMH treatment on the production of IL-2 by C. parvum-injected mice.

The con A-induced IL-2 activity of splenocytes from UDMH-C. parvum-treated mice was significantly greater than that of splenocytes from mice treated with C. parvum alone after 48 hours of stimulation by con A, but substantially less than that of normal mouse splenocytes (Table 10). The IL-2 production from splenocytes incubated with con A for 24 hours was not significantly different between UDMH-C. parvum-treated mice and mice treated with C. parvum alone; both were significantly lower than normal mouse splenocyte IL-2 production (Table 9).

4. Significance:

These results lend further credence to the conclusion drawn from previous experiments that the macrophage is a major target cell of UDMH. When considered with the effects of UDMH on chemiluminescence, they would imply that the activated macrophage may be the most sensitive to UDMH, and since activated macrophages suppress many lymphocyte functions, this conclusion is compatible with the immunoenhancement effects of UDMH.

F. Evaluation of the effects of UDMH on the immunosuppressive properties of activated macrophages and hydrogen peroxide (note: these experiments have just been started, so no results are available).

1. Rationale:

To determine if UDMH is truly influencing the activated macrophage in C. parvum treated mice, in vitro experiments are planned in which macrophages from C. parvum-treated mice will be exposed to UDMH, then assayed for their suppressive effect when added to LBT cultures. These experiments should further delineate if a major target cell of UDMH immunomodulation is the activated macrophage, as previous experiments suggest.

One mechanism of suppression of the LBT by activated macrophages is purported to be cell membrane lipid peroxidation induced by hydrogen peroxide (which is produced by activated macrophages during the respiratory burst) (Zoschke and Messner, Clin Res 30:544A, 1982). To determine if inactivation of hydrogen peroxide is a mechanism by which UDMH reverses the suppressive effects of C. parvum-activated macrophages, UDMH and suppressive but not cytotoxic amounts of hydrogen peroxide will be added to normal splenocytes in the LBT assay.

2. Methods:

a. In vitro effects of UDMH on C. parvum-activated macrophage suppression of LBT: activated peritoneal macrophages will be harvested from C. parvum-treated mice 10-14 days following C. parvum administration; they will be preincubated with PBS (control) or UDMH, then added in varying numbers to normal or T-cell-enriched (by passage through a nylon wool column) splenocytes from normal mice. An LBT assay with con A will be performed as previously described. UDMH or PBS will also be added to the combined cell populations during the LBT assay.

b. Effects of UDMH on hydrogen peroxide-induced suppression of LBT: Hydrogen peroxide and UDMH or PBS will be added to splenocytes from normal mice in the LBT assay. Also, splenocytes will be preincubated with UDMH, then washed and assayed for LBT in the presence of hydrogen peroxide.

III. Written Publications (cumulative list)

A. Suppression of mitogen-induced blastogenesis of feline lymphocytes by in vitro incubation with carcinogenic nitrosamides. Tarr, M.J. and Olsen, R.G. Immunopharmacology 2:191-199, 1980.

B. Differential effects of hydrazine compounds on B- and T-cell immune function. Tarr, M.J. and Olsen, R.G. AGARD Conference Proceedings No. 309, Toxic Hazards in Aviation, B3-1-7, 1981.

C. In vivo and in vitro effects of 1,1-dimethylhydrazine on selected immune functions. Tarr, M.J., Olsen, R.G. and Jacobs, D.L. Immunopharmacology 4:139-147, 1982.

D. Comparison of in vitro and in vivo immunotoxicology assays. Tarr, M.J., Olsen, R.G. and Jacobs, D.L. Annals N.Y. Acad. Sci. 407:469-471, 1983.

E. Species variation in susceptibility to methylnitrosourea-induced immunosuppression. Tarr, M.J. and Olsen, R.G. J. Env. Path. Toxicol. Oncol. 6:261-269, 1985.

F. Effects of 1,1-dimethyl-hydrazine on immunosuppression in mice treated with Corynebacterium parvum. Bauer, R.J., Tarr, M.J., and Olsen, R.G., submitted to Intl. J. Immunopharm. 1986.

G. Modulation of macrophage functions by 1,1-dimethylhydrazine. Tarr, M.J., Olsen, R.G., Bowen, B.L., Fertel, R.H. Submitted to Immunopharm., 1986.

H. The effects of 1,1-dimethylhydrazine on the murine allogeneic mixed lymphocyte response. McKown, B., Tarr, M.J. and Olsen, R.G. In preparation. To be submitted to Intl. J. Immunopharm.

I. Chemical alteration of host susceptibility to viral infection. Tarr, J.J. In: Comparative Pathobiology of Viral Diseases, R.G. Olsen, S. Krakowka, and J.R. Blakeslee, Jr., ed. pp. 47-55, 1985.

IV. Professional Personnel Associated with Research Effort

Richard G. Olsen, Ph.D.,Principal Investigator

Departments of Veterinary Pathobiology, Microbiology (College of Biological Sciences), and Comprehensive Cancer Center, The Ohio State University, Columbus, Ohio 43210.

Melinda J. Tarr, D.V.M., Ph.D., Co-Investigator

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Brian L. Bowen, B.S., M.S., Graduate Research Associate

Mr. Bowen received his Master of Science degree in August, 1985, and was supported by this contract. Thesis title: "The In Vitro and In Vivo Effects of 1,1-Dimethylhydrazine on the Chemiluminescent

Response of Activated Murine Peritoneal Macrophages." He is currrently a veterinary student in The Ohio State University College of Veterinary Medicine.

Richard M. Bauer, B.S., M.S. Graduate Research Associate.

Mr. Bauer is currently working towards his Doctor of Philosophy degree. He was partially supported by this contract until its termination and will be partially supported by the upcoming grant when it commences April 15.

V. Oral Presentations July, 1984 to December, 1985).

A. Possibility of Adverse Side Effects of Immunomodulators. Tarr, M. and Olsen, R. Presented at Charter Conference, Inter-American Society for Chemotherapy, Inc., Tampa, Florida, Dec. 1984.

B. Suppression of Murine Peritoneal Macrophage Chemiluminescence by 1,1-Dimethylhydrazine. Intl. J. Immunopharm. 7:374, 1985. Presented at 3rd Intl. Conference, Florence, Italy, May, 1985.

C. 1,1-Dimethylhydrazine Suppresses Activated Macrophage Functions. Tarr, M.J., Bauer, R.M., Bowen, B.L., and Olsen, R.G. Presented at 2nd Annual Conference, Inter-American Society for Chemotherapy, Tampa, Florida, Dec. 1985.

D. UDMH <u>In Vivo</u> Regulatory Role in Macrophage Function. Bauer, R.M., Tarr, M.J., and Olsen, R.G. Presented at 2nd Annual Biomedical High Technology Conference, Columbus, Ohio, Nov. 1985.

Table 1. In vitro Effects of UDMH on the Allogeneic Mixed Lymphocyte Reaction

Concentration UDMH (μg/ml)	UDMH present throughout MLR	UDMH pretreatment of responders	UDMH pretreatment of stimulators
0 (control)	35,951 ± 14,725	34,805 ± 6,904	36,177 ± 15,327
5	43,400 ± 11,366[a]	34,671 ± 5,680	37,093 ± 13,367
10	40,636 ± 12,701[a]	36,485 ± 5,814	37,747 ± 16,778
25	33,923 ± 11,067	33,986 ± 6,663	39,669 ± 14,947[a]
50	20,934 ± 7,475[b]	34,529 ± 6,858	43,012 ± 17,501[a]
75	13,939 ± 6,492[b]	34,576 ± 4,072	43,529 ± 22,169[a]
100	ND[c]	ND	41,152 ± 6,398[a]

[a]Significantly greater than control according to Dunnett's Multiple Comparison when = .05.

[b]Significantly less than control according to Dunnett's Multiple Comparison when = .05.

[c]ND = not done.

Table 2. Effects of UDMH Treatment on Time of Peak Chemiluminescence of PEC.

Concentration of UDMH (µg/ml)	Time of peak response (minutes) (mean ± standard deviation)
0 (control)	17.1 ± 5.8
1	14.4 ± 3.9
5	14.4 ± 3.9
10	14.4 ± 4.2
25	15.0 ± 3.2
50	14.3 ± 3.5
100	11.9 ± 3.7[a]
200	10.7 ± 1.9[b]

[a] $p = 0.030$ (Student "t" test; $n = 6-0$)

[b] $p \leq 0.010$

Table 3. Effects of UDMH Treatment on Murine Allogeneic Lymphocyte Response.[a]

Dose UDMH (mg/kg)	Type of Treatment		
	Treated R[b]	Treated S[b]	Treated R & S [b]
0 (control)	24,100	24,100	24,100
5	56,320[c]	38,240[c]	51,160
10	74,875[c]	28,730[c]	58,615[c]
25	42,720[c]	43,025[c]	42,105[c]
50	53,660[c]	42,575[c]	40,545[c]
75	23,080	47,230[c]	35,400[c]
100	ND	59,585[c]	ND

[a]C57/B16 mice provided responder cells; Balb/c mice provided stimulator cells. Response is expressed in counts per minute (see "Methods" in text).

[b]R = responder mice; S = stimulator mice.

[c]Significantly different from control at 5% level (Dunnett's Multiple Comparison test).

Table 4: Effects of UDMH on Interleukin 1 Production[a]

[UDMH] (ug/ml)	Time UDMH present in $P388_{D1}$ cultures t = 0-5 hrs	t = 5-29 hrs
0	28,019 ± 6,67 (s.e.m.)	36,537 ± 8,634
10	22,963 ± 5,338	39,627 ± 1,373
20	21,381 ± 5,260	35,656 ± 7,258
25	28,072 ± 5,690	57,892 ± 11,545
50	21,680 ± 3,204	52,960 ± 6,873
100	29,096 ± 5,783	54,216 ± 7,970

[a]Evaluated by measurement (counts per minute) of tritiated thymidine uptake by C3H/HeJ thymocytes in the presence of $P388_{D1}$ supernatant and phytohemagglutinin (see section C2).

Table 5. Effects of UDMH on IL-1 Activity[a]

[UDMH] (μg/ml)	Proliferation of C3H/HeJ Thymocytes (counts/minute)	Viability of C3H/HeJ Thymocytes
0	172,306 ± 3,283	89%
10	117,009 ± 5,108	91%
20	91,020 ± 7,822	93%
25	70,089 ± 5,162	87%
50	32,168 ± 4,833	86%
100	284 ± 54	82%

[a]UDMH was added to C3H/HeJ thymocytes with a standard source of IL-1 and Pha. Tritiated thymidine uptake and viability of thymocytes was determined after 72 hours of culture.

Table 6. Effect of UDMH on IL-2 Production by Murine Splenocytes.

Splenocyte Treatment[a]	cpm[b] of CTLL-20 cells	% Control
Con A (Control)	38,482	---
Con A + 50 µg/ml UDMH	41,225	105%
Con A + 100 µg/ml UDMH	31,388	82%

[a]Splenocytes were incubated for 24 hours, then the supernatant was harvested and assayed for IL-2 activity on CTLL-20 cells.

[b]cpm = counts per minute.

Table 7. Effects of UDMH on IL-2 Activity[a]

Concentration UDMH (µg/ml)	Percent of Control Response[b]
0 (control	(100%)
5	110.6%
10	136.6%
15	120.3%
20	108.0%
25	140.7%
30	94.8%
35	64.7%
40	79.1%
45	45.6%
50	28.9%

[a]Determined by measurement of the proliferative response of CTll-20 cells in the presence of a standardized common source of IL-2, with or without UDMH.

[b]Expressed as

$$\frac{\text{counts per minute of UDMH-treated CTLL-20 cells}}{\text{counts per minute of untreated CTLL-20 cells}} \times 100$$

Table 8. Effects of UDMH on the IL-2 Molecule[a]

Incubation temperature	Concentration UDMH (µg/ml)	IL-2 Activity
23°C	0	146,909 ± 2,350[b]
23°C	100	145,262 ± 4,352
37°C	0	144,755 ± 4,169
37°C	100	148,519 ± 8,751

[a]Medium containing known IL-2 activity was incubated for 24 hours with or without 100 µg/ml UDMH at the indicated temperatures, then assayed for IL-2 activity using CTLL-20 cells.

[b]Expressed as counts per minute ± standard deviation.

Table 9. Effects of UDMH Treatment on Con A-Induced Lymphocyte Blast Transformation Response of Splenocytes from Corynebacterium Parvum-Treated Mice[a]

Treatment	Counts per minute	
	24 hours	48 hours
Control (no treatment	17,193 ± 2,050 (s.e.m.)[b]	93,165 ± 6,400
C. parvum	1,931 ± 640[c]	2,735 ± 500[c]
C. parvum + 25 mg/kg UDMH	13,621 ± 1,250[d]	15,202 ± 8,650[e]
C. parvum + 50 mg/kg UDMH	13,028 ± 2,050[d]	14,749 ± 5,100[e]
C. parvum + 100 mg/kg UDMH	13,629 ± 1,850[d]	18,933 ± 5,500[e]

[a]Splenocytes were cultured with con A and evaluated for Tdr uptake after 24 and 48 hours.

[b]s.e.m. = standard error of the mean.

[c]Significantly less than control at $p < .001$.

[d]Significantly greater than C. parvum alone at $p < 0.001$; not significantly different from control.

[e]Significantly greater than C. parvum alone at $p < 0.001$; significantly less than control at $p < 0.001$.

Table 10. Effects of UDMH Treatment on Interleukin 2 Production by Splenocytes from Corynebacterium Parvum-Treated Mice[a]

Treatment	Counts per minute	
	24 hours	48 hours
Control (no treatment)	59,714 ± 600 (s.e.m.)[b]	57,410 ± 750
C. parvum	9,065 ± 1,000[c]	7,423 ± 1,150[c]
C. parvum + 25 mg/kg UDMH	12,346 ± 1,400[c]	20,105 ± 3,050[c,d]
C. parvum + 50 mg/kg UDMH	22,945 ± 7,100	13,563 ± 1,550[c,d]
C. parvum + 100 mg/kg UDMH	16,682 ± 2,600 [c]	12,297 ± 950[c,d]

[a]Interleukin 2 activity determined by proliferative response of CTLL-20 cells when exposed to supernatants of splenocytes incubated with concanavalin A for either 24 or 48 hours.

[b]s.e.m. = standard error of the mean.

[c]Significantly less than control at $p < 0.002$.

[d]Significantly greater than C. parvum alone at $p < 0.04$.

Figure 1. Effects of UDMH on PGE_2 synthesis by adherent resident peritoneal cells. Cells were culture in the presence of UDMH and 0.1 ug/ml lipopolysaccharide for 24 hours. Control response = 100%. Vertical bars represent standard error of the mean (n = 17-19).

[a] $p \leq 0.025$ (paired "t" test)

[b] $p \leq 0.001$ (paired "t" test)

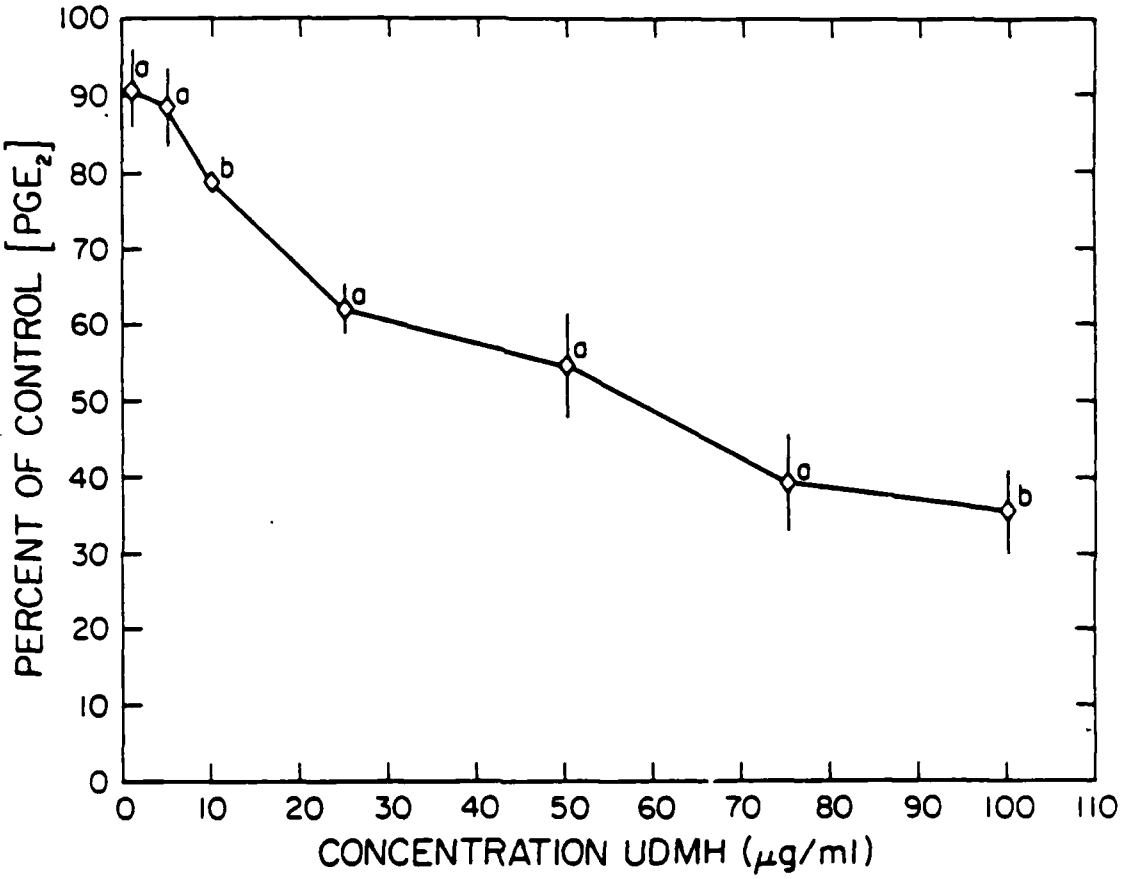
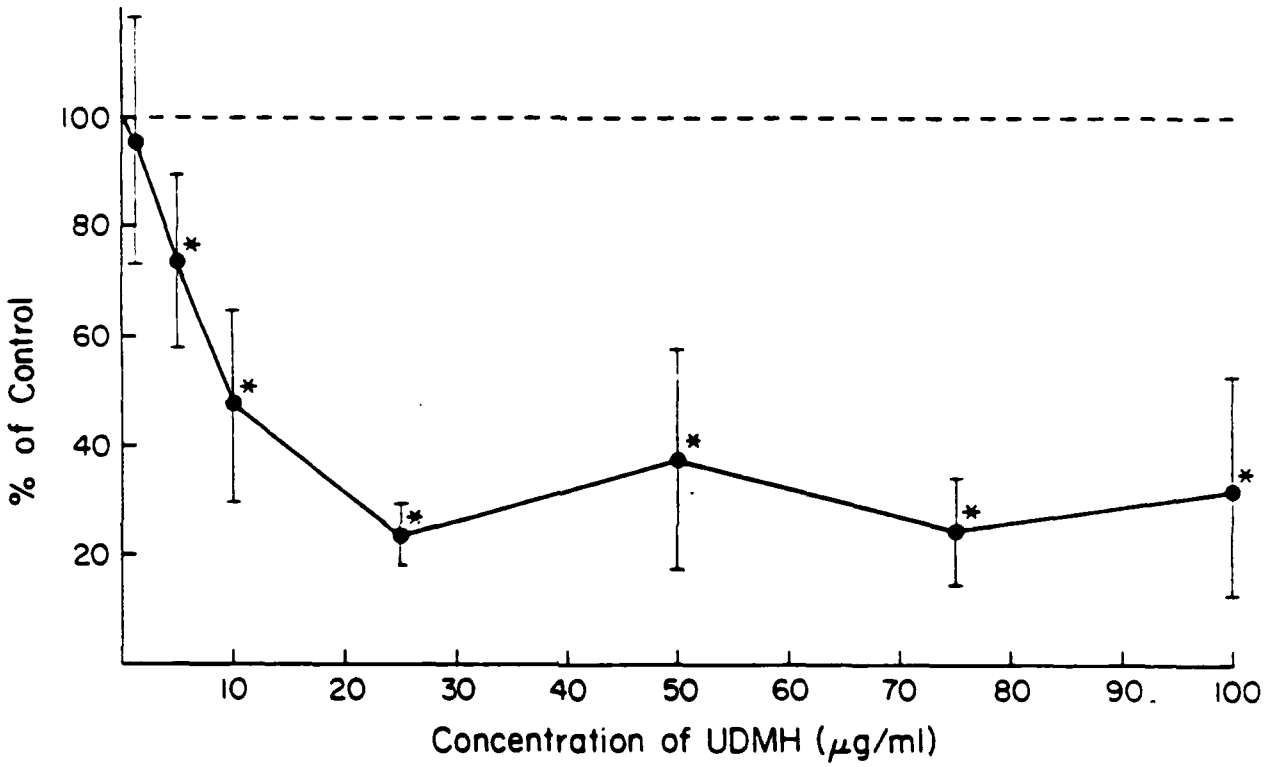

PERCENT OF CONTROL [PGE2]
CONCENTRATION UDMH (μg/ml)
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
50
60
70
80
90
100
110
a
a
b
a
a
b

Figure 2. Effects of UDMH on the chemiluminescent response of peritoneal macrophages activated *in vitro* by *C. parvum*. Asterisk indicates responses which were significantly less than control response (100%) at $p \leq .001$ (paired "t" test). Vertical bars represent standard error of the mean ($n = 6-9$).


100
80
60
40
20
% of Control
10
20
30
40
50
60
70
80
90
100
Concentration of UDMH (μg/ml)

END

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